Updated Search Note

L5: Entry 6 of 9 File: USPT Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6299883 B1
TITLE: Tarf

Brief Summary Text (7):
A target that would be useful in developing such factors are teichoic acids, which are polymers found in the cell walls of Gram-positive bacteria. Teichoic acids have been shown to be essential for viability and normal morphogenesis of several Gram-positive species. CDP-glycerol:poly(glycerophosphate) glycerophospho transferase is a key enzyme in the wall teichoic acids biosynthetic pathway. The gene is named tarF in S.aureus, which makes the ribitol-based polymer, and tagF in Bacillus subtilis, which make the glycerol-based polymer. The enzyme adds CDP-activated glycerol in the final step of synthesis of the lipid-linked linkage unit on which the polymer is synthesised. Mutation of this gene has been shown to be lethal in Bacillus subtilis and Staphylococcus aureus and will prevent the synthesis of wall teichoic acids, which are essential for the viability of Gram-positive cells. Although there are many types of wall teichoic acids in bacteria, the linkage unit structure is conserved in most species tested, and thus inhibition of this enzyme is a valid antibacterial strategy.

0074] In gram-positive bacteria, such as Corynebacterium glutamicum, the cytoplasmic membrane is covered by a multi-layered peptidoglycan, which ranges from 20-80 nm in thickness (see, e.g., Lengeler et al. (1999) Biology of Prokaryotes Thieme Verlag: Stuttgart, p. 913-918, p. 875-899, and p. 88-109 and references therein). The gram-positive cell wall also contains teichoic acid, a polymer of glycerol or ribitol linked through phosphate groups. Teichoic acid is also able to associate with amino acids, and forms covalent bonds with muramic acid. Also present in the cell wall may be lipoteichoic acids and teichuronic acids. If present, cellular surface structures such as flagella or capsules will be anchored in this layer as well.

Detail Description Table CWU:

Summary of Invention Paragraph:
[0004] Bacillus pumilus strain Sh 18 ("B. pumilus Sh18") is a nonpathogenic, enteric, gram-positive bacterium. It has been reported that this bacterium produces a cell wall polysaccharide (sometimes referred to as a teichoic acid) that cross-reacts serologically with the capsular polysaccharide (CP) of Hib (Argman et al. (1974), J Immunol. 112, 649-55). This cross reactivity has been attributed to poly (ribotol phosphate) known to be present in cell wall associated teichoic acids of at least some bacilli (Kojima et al. (1985) J. Bacteriol 161, 299-306). No cross-reactivity, however, was observed with Hia CP, which is structurally similar to Hib CP, and which also contains ribotol phosphate in its subunit. It has been suggested that enteric, non-pathogenic gram positive bacteria, such as B. pumilus Sh18, may serve as a source of natural immunity against Hib in children over 6 years old and in adults (Bradshaw et al. (1971), Lancet 1095-6). Cross reactivity of B. pumilus Sh18 cell wall polysaccharide (CWP) with bacterial cell surface polysaccharides other than Hib has not been suggested or reported previously.

Detail Description Paragraph:

Schneerson et al. (1980), J. Exp Med, 152, 361-76 and Vann et al. (1976), Infect Immun. 13, 1654-62). Briefly, CP and CWP were isolated from culture supernatant by precipitation with 0.1% Cetavlon and purified by enzyme treatment and cold phenol extraction, followed by separation on a Sepharose CL-6B gel filtration column (0.2 M NaCl as eluent). The identity of H. influenzae types a and b CP was confirmed by precipitation in double immunodiffusion with the type specific burro serum against H. influenzae types a and b (Myerowitz et al. (1973), Infect Immun, 8, 896-900) and by nuclear magnetic resonance (NMR) spectroscopy by comparison to the published spectra (Lemercinier et al. (2000) Biologicals 28, 175-183; Zon et al. (1983) Carbohydr. Res. 114, 103-121. The CWP of S. aureus type 5 was further separated from its CP by Sephadex DEAE chromatography. Fractions showing a positive reaction with rabbit anti-S. aureus teichoic acid serum and a negative reaction with rabbit anti-S. aureus type 5 CP were collected. S. epidermidis CWP was precipitated with 80% ethanol from culture supernatant, treated with enzymes and chromatographed on a BioGel P100 column equilibrated with PBS. Anti-S. epidermidis sera were prepared by intravenous immunization of rabbits with acetone-dried bacterial cells as described (Alexander et al. (1946) J Immunol 54, 207-214). B. pumilus Sh18 CWP was further purified by passage through Sephadex DEAE chromatography (0.1-2.0 M NaCl gradient). Fractions demonstrating positive reactions with H. influenzae type b antiserum were collected and analyzed.

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L8: Entry 4 of 23 File: PGPB Jul 21, 2005

DOCUMENT-IDENTIFIER: US 20050158346 A1
TITLE: Antimultiorganism Glycoconjugate vaccine

Abstract Paragraph:
The present invention relates, e.g., to a glycoconjugate composition comprising one or more polysaccharide types from a cell wall polysaccharide preparation from B. pumilus Sh 18, or variants thereof. Also disclosed are antibodies generated against the glycoconjugates, and methods of using the glycoconjugates and antibodies. An antimultiorganism vaccine which reacts against at least Haemophilus influenzae type a, Haemophilus influenzae type b, Staphylococcus aureus, and Staphylococcus epidermidis, is disclosed.

Summary of Invention Paragraph:
[0003] Bacterial pathogens are responsible for variety of diseases worldwide. Among these pathogens are Haemophilus influenza type a ("Hia"), Haemophilus influenza type b ("Hib"), Staphylococcus aureus ("S. aureus") and Staphylococcus epidermidis ("S. epidermidis"). H. influenza type a has been reported to cause infections in Africa (Wall et al. (1986), Bull World Health Organ 64, 553-8; Wall et al. (1985), Lancet 845) and other countries (Rutherford et al. (1984), Ped Inf Dis 3, 575-77). Haemophilus influenza type b causes serious diseases, including mental retardation, which have the highest incidence in infancy and childhood. Staphylococcus aureus causes several diseases, the most frequent and serious of which are bacteremia and its complications in hospitalized patients. In particular, S. aureus can cause wound infections and infections associated with catheters and prosthetic devices. Serious infections associated with S. aureus bacteremia include osteomyelitis, invasive endocarditis and septicemia. Staphylococcus epidermidis causes disease primarily in patients with impaired host defenses or altered microbial flora, and is common in newborns. In particular, S. epidermidis can cause urinary tract infections, infections associated with IV catheters, meningitis in patients with subacute bacterial endocarditis, and it can cause mastitis in dairy animals.

Summary of Invention Paragraph:
[0004] Bacillus pumilus strain Sh 18 ("B. pumilus Sh18") is a nonpathogenic, enteric, gram-positive bacterium. It has been reported that this bacterium produces a cell wall polysaccharide (sometimes referred to as a teichoic acid) that cross-reacts serologically with the capsular polysaccharide (CP) of Hib (Argman et al. (1974), J Immunol. 112, 649-55). This cross reactivity has been attributed to poly (ribotol phosphate) known to be present in cell wall associated teichoic acids of at least some bacilli (Kojima et al. (1985) J. Bacteriol 161, 299-306). No cross-reactivity, however, was observed with Hia CP, which is structurally similar to Hib CP, and which also contains ribotol phosphate in its subunit. It has been suggested that enteric, non-pathogenic gram positive bacteria, such as B. pumilus Sh18, may serve as a source of natural immunity against Hib in children over 6 years old and in adults (Bradshaw et al. (1971), Lancet 1095-6). Cross reactivity of B. pumilus Sh18 cell wall polysaccharide (CWP) with bacterial cell surface polysaccharides other than Hib has not been suggested or reported previously.

Detail Description Paragraph:
[0012] The present inventors have determined the structure of a cell wall

polysaccharide ("CWP") composition from the enteric, gram positive, non-pathogenic bacterium, Bacillus pumilus Sh 18. The CWP composition comprises three polysaccharide (PS) components: (1) a major component which comprises a 1,5-poly (ribitol phosphate); (2) a major component which comprises 1,3-poly(glycerol phosphate) that is partially substituted by 2-acetamido-2-deoxy-.alpha.-galactose (about 14%) and 2-acetamido-2-deoxy-.alpha.-glucose (about 7%) on position C-2; and (3) a minor component which comprises a poly(2-acetamido-2-deoxy-.beta.-glucosy- 1-1.fwdarw.4-ribitol phosphate) with the phosphodiester bonds located between C-1 of ribitol and C-3 of 2-acetamido-2-deoxy-.beta.-glucose.

Detail Description Paragraph:
[0014] Also disclosed are several types of glycoconjugate preparations which comprise components of the above B. pumilus Sh 18 cell wall polysaccharide composition. One of these glycoconjugate preparations is made by activating the terminal phosphate residues of the polysaccharides, e.g., with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide- -HCl (EDAC), in order to facilitate their binding to a protein or peptide. Surprisingly, such a glycoconjugate preparation cross-reacts with a wide spectrum of sera prepared against bacterial capsular polysaccharides, including those from at least the four pathogenic bacteria: Haemophilus influenza type a (Hia), Haemophilus influenza type b (Hib), Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis). The glycoconjugate preparation also reacts with sera prepared against the non-pathogenic bacteria, B. pumilus Sh18, itself, and against the closely related nonpathogenic bacterium, B. pumilus Sh17. Those reactivities with non-pathogenic bacteria are generally not referred to further in the present disclosure, but are to be understood to occur.

Detail Description Paragraph:
[0017] The present invention relates, e.g., to a glycoconjugate preparation (e.g., composition), comprising one or more polysaccharides from a cell wall polysaccharide preparation from B. pumilus Sh 18, wherein one or more of the polysaccharides is bound to a protein or peptide. Embodiments of this glycoconjugate preparation react with at least an antiserum generated against S. epidermidis, H. influenza type a (Hia), and/or S. aureus, and, optionally, with an antiserum generated against H. influenza type b (Hib). Preferably, the glycoconjugate preparation reacts with antisera generated against at least all four of those pathogenic bacteria. Furthermore, embodiments of this glycoconjugate preparation induce antibodies cross-reactive with at least cell wall polysaccharide (CWP) of S. aureus and/or S. epidermidis and/or with CP of Hia and, optionally, with CP of Hib. Preferably, the glycoconjugate preparation induces antibodies cross-reactive with at least CP or CWP of all four of those pathogenic bacteria.

Detail Description Paragraph:
[0018] In other embodiments of the invention, the glycoconjugate preparation comprises a poly(ribitol phosphate) (e.g., a 1,5-poly(ribitol phosphate)) and/or a poly(glycerol phosphate) (e.g., a 1,3-poly(glycerol phosphate)) and, optionally, a poly(2-acetamido-2-deoxy-.beta.-glucosyl-1- .fwdarw.4-ribitol phosphate) with the phosphodiester bonds located between C-1 of ribitol and C-3 of 2-acetamido-2-deoxy-.beta.-glucose. In some embodiments, the 1,3-poly(glycerol phosphate) is partially substituted by 2-acetamido-2-deoxy-.beta.-galactose and/or 2-acetamido-2-deoxy-.alpha.-g- lucose on position C-2, e.g., about 14% of the 1,3poly(glycerol phosphate) is substituted by 2-acetamido-2-deoxy-.beta.-galactose, and/or about 7% is substituted by 2-acetamido-2-deoxy-.alpha.-glucose.

Detail Description Paragraph:
[0019] Another aspect of the invention is a glycoconjugate preparation that consists essentially of (a) a poly(ribitol phosphate) (e.g., an unsubstituted poly (ribitol phosphate), such as an unsubstituted 1,5-poly(ribitol phosphate)) or (b) a poly(glycerol phosphate) (e.g., an unsubstituted poly(glycerol phosphate), such as an unsubstituted 1,3-poly(glycerol phosphate)), wherein the polysaccharide (a) or

(b) is bound to a protein or peptide.

Detail Description Paragraph:
[0022] (b) a 1,3-poly(glycerol phosphate (e.g., an unsubstituted or partially substituted 1,3-poly(glycerol phosphate)); or

Detail Description Paragraph:
[0023] (c) a poly(2-acetamido-2-deoxy-.alpha.-glucosyl-1.fwdarw.4-ribitol phosphate) with the phosphodiester bonds located between C-1 of ribitol and C-3 of 2-acetamido-2-deoxy-.beta.-glucose. In one embodiment, the 1,3-poly(glycerol phosphate) is partially substituted on position C-2 by 2-acetamido-2-deoxy-.beta.-galactose and/or 2-acetamido-2-deoxy-.alpha.-g- lucose, e.g., about 14% of the 1,3-poly(glycerol phosphate) is substituted by 2-acetamido-2-deoxy-.beta.-galactose, and/or about 7% is substituted by 2-acetamido-2-deoxy-.alpha.-glucose. In one embodiment, the molar ratio of components (a), (b) and (c) is approximately 56:34:10. The precise ratio of the three components is not critical, and can vary somewhat depending on, e.g., growth conditions. Any ratio which provides a glycoconjugate having functional properties according to the invention (e.g., the ability to react with an antiserum generated against S. epidermidis, Hia, and/or S. aureus, and, optionally, Hib) is within the scope of the invention.

Detail Description Paragraph:
[0025] Alternatively, one or more of the polysaccharides may be bound to peptides or proteins via a linkage between a terminal phosphate group of the polysaccharide and a reactive amino group of the peptide or protein. For example, the reactive amino group may be in an adipic dihydrazide (ADH) group that has been used to derivative the peptide or protein. When this type of linkage is present, the glycoconjugate generally reacts with antisera generated against at least S. epidermidis, Hia, and/or S. aureus, and, optionally, Hib (preferably against at least all four of those pathogenic bacteria); and/or the glycoconjugate induces antibodies cross reactive with at least cell wall polysaccharide (CWP) of S. aureus and/or S. epidermidis and/or with capsular polysaccharide (CP) of Hia and, optionally, with CP of Hib. Preferably, the glycoconjugate induces antibodies cross reactive with at least CWP or CP of all four of those pathogenic bacteria.

Detail Description Paragraph:
[0029] Another aspect of the invention is glycoconjugate made with an isolated poly (glycerol phosphate) from the B. pumilus Sh18 cell wall polysaccharide. A skilled worker will be aware of conventional methods for isolating and, if desired, purifying, such a poly(glycerol phosphate). For example, the cell wall polysaccharide preparation can be oxidized to release the poly(glycerol phosphate) component, which can then be isolated from other components of the preparation. Alternatively, a poly(glycerol phosphate) can be generated synthetically, using conventional procedures. Such a synthetic poly(glycerol phosphate) is preferably unsubstituted. An unsubstituted polysaccharide would be expected to induce antibodies that are cross-reactive with poly(glycerol phosphate)-containing polysaccharides from a variety of bacteria, even if those bacteria contain poly (glycerol phosphate)-containing polysaccharides which are substituted with different substituents than the poly(glycerol phosphate)-containing polysaccharide of the B. pumilus Sh18 cell wall. Alternatively, the synthetic poly(glycerol phosphate) may be substituted, for example in the manner in which the poly(glycerol phosphate)-containing polysaccharide of the B. pumilus Sh18 cell wall is substituted.

Detail Description Paragraph:
[0032] Methods of preparing glycoconjugates with the isolated poly(ribitol phosphate) or poly(ribitol phosphate) chains of the invention are conventional, as are methods to test whether a conjugate of interest elicits the desired specificity and/or degree of immunogenicity. One of skill in the art will recognize a variety of types of glycoconjugates that can be generated. For example, one can

synthetically add a linker having an available amino group to a synthetic poly (ribitol phosphate) or poly(glycerol phosphate) chain, and conjugate this to a protein having an available carboxyl group, or vice versa. A wealth of suitable linker molecules will be evident to the skilled practitioner. Preferably, a glycoconjugate of the invention comprises about 5-20 polysaccharide chains per each protein or peptide molecule. For guidance in some synthetic methods of preparing conjugates, see Pozsgay et al. (1999) Proc. Natl. Acad. Sci. USA 96, 5194-5197; Schneerson et al, (2003) Proc. Natl. Acad. Sci. USA 100, 8945-8950.

Detail Description Paragraph:
[0034] In one embodiment, the antibody is a polyclonal antibody. A polyclonal antibody of the invention may be prepared against a glycoconjugate in which one or more of the polysaccharides are bound to peptides or proteins by a linkage via a terminal phosphate group of the polysaccharide. For example, the linkage may be formed by activating a terminal phosphate group of the polysaccharide (e.g., with EDAC) and then binding the activated phosphate to a reactive amino group in a suitable peptide or protein. A polyclonal antibody prepared in this manner preferably comprises species of antibody binding sites that react with polysaccharides of the cell walls of Hia, S. aureus, and/or S. epidermidis and, optionally, Hib (preferably against all four of those bacteria).

Detail Description Paragraph:
[0035] Alternatively, the polyclonal antibody may be prepared against a glycoconjugate in which one or more of the polysaccharides are bound to peptides or proteins by a linkage via a hydroxyl group of the polysaccharide. Preferably, the polyclonal antibody comprises species of antibody binding sites that react with polysaccharides of S. epidermidis (and/or B. pumilus Sh18 and/or Sh17), but not Hib, Hia, or S. aureus.

Detail Description Paragraph:
[0036] The invention also relates to an antibody preparation comprising a collection (mixture) of one or more monoclonal antibodies, which cross react with CP or CWP of Hia, S. aureus, and/or S. epidermidis, and, optionally, a monoclonal antibody specific for a cell wall polysaccharide of Hib. Preferably, the collection of monoclonal antibodies comprises species of antibody binding sites that react with CP or CWP of all four bacteria. The monoclonal antibodies can be generated, e.g., against a glyconjugate in which the linkage between the polysaccharide and the peptide or protein is via an activated phosphate group in the polysaccharide. Other suitable monoclonal antibodies can be generated against glycoconjugates made with isolated or purified poly(glycerol phosphate) or poly(ribitol phosphate).

Detail Description Paragraph:
[0037] The invention also relates to an antibody, either polyclonal or monoclonal, which is generated against a B. pumilus Sh18 CWP. Such an antibody preferably contains antibodies that react with the cell wall material of Hib and/or S. epidermidis (and/or B. pumilus Sh18 or Sh17), but does not contain antibodies that are cross-reactive with Hia, or S. aureus.

Detail Description Paragraph:
[0039] Another aspect of the invention is a method for eliciting (inducing) an immune response in a subject, comprising administering to the subject an immunostimulatory-effective amount of a glycoconjugate preparation of the invention. The subject may be, e.g., a subject (e.g., a patient) who is likely to be exposed to (infected by), or who has already been exposed to (infected by), or is suspected of having been exposed to (infected by), Hia, S. aureus, and/or S. epidermidis and, optionally, Hib. In embodiments of the invention, the immune response is protective against infection by the bacteria Hia, S. aureus, and/or S. epidermidis and, optionally, Hib; in one embodiment, the immune response is protective against infection by all four of those bacteria. In one embodiment, the method further comprises detecting resistance of the subject to infection by Hia,

S. aureus, and/or S. epidermidis and, optionally, Hib; in one embodiment, the method comprises detecting resistance of the subject to infection by all four of those bacteria.

Detail Description Paragraph:
[0041] Other aspects of the invention are directed to immunotherapy methods. For example, the invention relates to an immunotherapy method, comprising administering to a subject in need thereof an effective amount of an antibody of the invention. Another embodiment of the invention is a hyperimmune globulin composition generated against a glycoconjugate preparation of the invention. The hyperimmune composition may comprise antibodies directed against cell wall components of Hia, S. aureus, and/or S. epidermidis and, optionally, Hib; or against cell wall components of all four of those bacteria. Another embodiment is a method for preparing an immunotherapy agent, comprising immunizing a subject with a glycoconjugate of the invention; collecting plasma from the immunized subject, and harvesting a hyperimmune globulin from the collected plasma. The hyperimmune globulin may contain antibodies directed against cell wall components of Hia, S. aureus, and/or S. epidermidis and, optionally, Hib; or against cell wall components of all four of those bacteria.

Detail Description Paragraph:
[0043] Suitable polysaccharide constituents of the glycoconjugate preparations of the invention include any of the polysaccharides discussed herein. The polysaccharides may be isolated from a B. pumilus Sh 18, or from another suitable organism. Suitable methods for isolating and, optionally, purifying the polysaccharides are conventional. See, e.g., Schneerson et al. (1980), J. Exp Med, 152, 361-76, or Vann et al. (1976), Infect Immun. 13, 1654-62, or the present Examples. Depending on the growth conditions and the method of preparation, the glycoprotein composition may comprise varying proportions of the three polysaccharide types (a), (b), and (c) noted above; and the polysaccharides may exhibit different amounts and/or structures of the noted substituents, compared to the naturally occurring polysaccharides in the bacterium. Any such polysaccharide variant is in accordance with the invention, provided that it retains a functional property in accordance with the invention (e.g., the ability to react with an antiserum generated against S. epidermidis, Hia, and/or S. aureus, and, optionally, Hib). Alternatively, the polysaccharides may be generated synthetically, using procedures that will be evident to a skilled worker.

Detail Description Paragraph:
[0052] As the Examples show, a glycoconjugate comprising a polysaccharide from a cell wall polysaccharide preparation from B. pumilus Sh 18, when prepared by the preceding method, exhibits much broader cross-reactivity than does a glycoconjugate made by the more conventional methods. A glycoconjugate made by conventional methods (e.g., Method 2) elicits antibodies that cross-react with S. epidermidis, but not with Hia, Hib, or S. aureus. By contrast, a glycoconjugate made by the new method described herein (Method 1) can elicit antibodies that react with at least all four of those bacteria. The fact that the glycoconjugate cross reacts with these particular, disparate, bacteria is unexpected and advantageous.

Detail Description Paragraph:
[0056] In one embodiment, the antibody preparation is a polyclonal antibody preparation. In another embodiment, the antibody preparation comprises a collection (mixture) of one or more monoclonal antibodies (e.g., mouse or human monoclonals). This preparation comprises species of antibody binding sites that react with polysaccharides of the cell walls of Hia, S. aureus, and/or S. epidermidis and, optionally, Hib (preferably against all four of those bacteria). Protocols for producing these antibodies are conventional and are described, e.g., in Ausubel, et al. (eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), Chapter 11; Bartal et al. (eds.), Methods of Hybridoma Formation, Humana Press, Clifton, N.J. (1988), pages 257-271; Vitetta et al.

(1982), Immunol. Rev. 62, 159-83; and Raso (1982), Immunol. Rev. 62, 93-117.

Detail Description Paragraph:
[0060] A monoclonal antibody (mAb) composition of the invention contains, within detectable limits, only one species of antibody combining site capable of effectively binding an epitope of a cell surface polysaccharide of one of the four bacteria noted above. Some monoclonal antibodies may be specific for an epitope that is common to all four of the cross-reacting polysaccharide types (e.g., a portion of a particular poly(ribitol phosphate)). Other monoclonal antibodies may be specific for an epitope that is present in only one of, or in two or three of, the cross-reacting polysaccharides. For example, a mAb produced in response to a poly(glycerol phosphate) of a B. pumilus Sh 18 cell wall polysaccharide may be specific for S. epidermidis (and for B. pumilus Sh18 and Sh 17), but not for bacteria whose cell wall components lack poly(glycerol phosphate), such as Hib, Hia and S. aureus. It is well within the ability of a skilled worker to determine the specificity of any mAb generated by a method of the invention, using conventional procedures. Various combinations of mAbs may be combined to produce a desired mixture (collection) of mAbs.

Detail Description Paragraph:
[0072] Among the diagnostic assays that can be performed with antibody preparations of the invention are screening assays. For example, antibodies can be used for screening a group of fresh bacterial isolates to distinguish between those which contain ribitol phosphate and those which contain glycerol phosphate as a subunit of their surface antigens. In this case, either ribitol phosphate or glycerol phosphate would be used as a group antigen. Similar assays are used for streptococcal group antigens.

Detail Description Paragraph:
[0074] Suitable subjects (e.g., patients in need of such treatment) include any animal that can generate an immune response, such as, e.g., mammals, including cat, mouse, rat, rabbit, guinea pig, hen, goat, donkey, burro, pig, horse, cow, non-human primate or, preferably, a human. For example, a suitable patient would be one who is likely to be exposed to (infected by), or who has already been exposed to (infected by), or who is suspected of having been exposed to (infected by), Hia, S. aureus, and/or S. epidermidis and, optionally, Hib. Conditions that can be prevented or treated by a method of the invention include any condition mediated by infection by one of the above-mentioned bacteria, including, e.g., the conditions discussed in the Background Information section above.

Detail Description Paragraph:
[0080] A B. pumilus Sh 18 antigen (polysaccharide or glycoconjugate) or antibody of the invention can be the active ingredient in a pharmaceutical composition, which further comprises a pharmaceutically acceptable carrier for the active ingredient. The pharmaceutical composition can be used, e.g., in a therapeutic or prophylactic application to confer passive immunity, or as a vaccine to induce a cellular immune response and/or production in vivo of antibodies which combat these bacterial infections. In this regard, a pharmaceutically acceptable carrier is a material that can be used as a vehicle for administering a medicament because the material is inert or otherwise medically acceptable, as well as compatible with the active agent, e.g., in the context of vaccine administration. Typical pharmaceutically acceptable carriers include, e.g., physiological saline, dextrose, glycerol, ethanol, or other injectable liquids. Additives customary in vaccines may also be present, for example stabilizers such as lactose or sorbitol, and adjuvants to enhance the immunogenic response such as aluminum phosphate, hydroxide, or sulphate and stearyl tyrosine. Other agents, such as antioxidants, preservatives, or solubilizing agents, may also be present.

Detail Description Paragraph:
[0086] The following bacterial strains were used in this study: Bacillus pumilus Sh

18 and Sh 17 (Meyrowitz et al. (1973), Infect Immun. 8, 896-900); Heamophilus influenzae type b (Eagan and Rab); Heamophilus influenzae type a strain Harding; Staphylococcus aureus type 5 strain Lowenstein; and Staphylococcus epidermidis RP-62A (ATCC 35981). Bacillus pumilus Sh 18 was obtained from the ATCC collection.

Detail Description Paragraph:
[0087] B. pumilus Sh 18 and Sh 17 were cultured in the ultrafiltrate of Tryptic Soy Broth (TSB), H. influenzae type a and b as previously reported (Schneerson et al., (1980), J Exp Med. 152, 361-76), S. aureus as described (Fattom et al. (1990), Infect Immun. 58, 2367-74), and S. epidermidis on chemically-defined medium (Hassain et al, (1991), J. Med Microbiol 34, 143-47).

Detail Description Paragraph:
[0089] Capsular polysaccharides (CP) from H. influenzae types a and b, and cell wall polysaccharides (CWP) from S. aureus and B. pumilus Sh18 and Sh17 were prepared as described in Schneerson et al. (1980), J. Exp Med, 152, 361-76 and Vann et al. (1976), Infect Immun. 13, 1654-62). Briefly, CP and CWP were isolated from culture supernatant by precipitation with 0.1% Cetavlon and purified by enzyme treatment and cold phenol extraction, followed by separation on a Sepharose CL-6B gel filtration column (0.2 M NaCl as eluent). The identity of H. influenzae types a and b CP was confirmed by precipitation in double immunodiffusion with the type specific burro serum against H. influenzae types a and b (Myerowitz et al. (1973), Infect Immun, 8, 896-900) and by nuclear magnetic resonance (NMR) spectroscopy by comparison to the published spectra (Lemercinier et al. (2000) Biologicals 28, 175-183; Zon et al. (1983) Carbohydr. Res. 114, 103-121. The CWP of S. aureus type 5 was further separated from its CP by Sephadex DEAE chromatography. Fractions showing a positive reaction with rabbit anti-S. aureus teichoic acid serum and a negative reaction with rabbit anti-S. aureus type 5 CP were collected. S. epidermidis CWP was precipitated with 80% ethanol from culture supernatant, treated with enzymes and chromatographed on a BioGel P100 column equilibrated with PBS. Anti-S. epidermidis sera were prepared by intravenous immunization of rabbits with acetone-dried bacterial cells as described (Alexander et al. (1946) J Immunol 54, 207-214). B. pumilus Sh18 CWP was further purified by passage through Sephadex DEAE chromatography (0.1-2.0 M NaCl gradient). Fractions demonstrating positive reactions with H. influenzae type b antiserum were collected and analyzed.

Detail Description Paragraph:
[0099] Mass spectra were recorded by using a JEOL SX102a magnetic sector instrument with xenon and 6 keV atoms to ionize samples from 3-nitrobenzyl alcohol or glycerol matrix.

Detail Description Paragraph:
[0113] The levels of antibodies were evaluated by ELISA using CovaLink plates (Nunc). In this assay, the terminal phosphate group of polysaccharide, in the presence of carbodiimide, forms a phosphoramide bond with secondary amino group exposed on the surface of the wells. Plates were coated with CP of H. influenzae types a and b and E. coli (negative control), and CWP of B. pumilus Sh 18 and Sh17, S. aureus type 5 and S. epidermidis RP-62A. Polysaccharides (5 .mu.g/ml) were dissolved in 10 mM 1-methylimidazol buffer (pH 7), and EDAC was added to a final concentration of 50 mM. The antigens were applied at 100 .mu.l per well and incubated at 37.degree. C. overnight. Plates were washed 6 times with 0.1% Brij 35-saline and blocked with 1% HSA in PBS for 1 h at room temperature. Twofold dilutions of the sera were made in 1% HSA-0.1% Brij 35-saline and incubated at 37.degree. C. for 4 h. Plates were washed, goat anti-mouse immunoglobulin G (IgG) conjugated to alkaline phosphatase were added and incubated at 37.degree. C. for 3 h. 4-nitrophenylphosphate (1 mg/ml in 1M Tris hydrochloride buffer, pH 9.8) and containing 0.3 mM MgSO.sub.4) was added, and the A.sub.405 was read after 30 min in an MR600 microplate reader (Dynatech). Some ELISAs were run using the avidin-biotin system. Murine monoclonal anti-Hib antibodies (0.52 mg/ml) were used as a standard for the ELISA. An inhibition ELISA was done by incubating mouse sera induced by

Sh18 CWP conjugate I, diluted to the concentration that gave an A.sub.405 absorption of 1.0, with 5 or 20 .mu.g of Hib, Hia, E. coli KI and E. coli K93 CP or Sh18, S. aureus, and S. epidermidis CWP/ml for 1 h at 37.degree. C. and overnight at 4.degree. C. The assay was then continued as above. Sera with and without inhibitor, at the same dilution, were compared. Percent inhibition was defined as follows: [1-(A.sub.405 of adsorbed serum)/(A.sub.405 of nonadsorbed serum)].times.100%.

Detail Description Paragraph:
[0116] Absorption of burro and anti-Hib serum with Sh18-CWP was done by adding 1 mg of CWP to 20 ml of serum (the ratio was based on the maximum precipitation in the quantitative precipitation assay). The solution was incubated at 37.degree. C. for 1 h and at 4.degree. C. for 2 days. The precipitate was removed by centrifugation at 37,000.times.g for 10 min and washed, and the composition was analyzed for ribitol and glycerol contents by GLC-MS.

Detail Description Paragraph:
[0117] B. pumilus Sh18 CWP was eluted from a Sepharose CL-6B column with a K.sub.d of 0.5 and from a Sephadex DEAE column by 0.48 M NaCl. In both cases, the CWP showed size and charge heterogeneity. The use of other sizing columns did not separate this peak into more components. This peak was divided into 5 fractions and compositional analysis of the eluted fractions showed that each contained ribitol, glycerol, and amino sugars, but in different molar ratios. The peak from the DEAE-Sephadex column that reacted with anti-Hib serum (FIG. 2), designated as Sh 18-CWP, was further characterized.

Detail Description Paragraph:
[0118] GLC-MS analysis of Sh18 CWP detected peracetylated derivatives of glycerol, ribitol, glyucosamine, and galactosamine (Table 1), and the CWP contained 10.7 wt % of phosphorous. HF hydrolysis (48% HF, 1 hat 60.degree. C.) released only ribitol and glycerol, whereas additional hydrolysis with HCl released amino sugars, indicating that they are bound by glycosidic linkages.

Detail Description Paragraph:
[0119] Treatment of the Sh18 CWP with HF (48%, 4.degree. C. for 48 h) followed by BioGel P-2 chromatography revealed low-molecular-mass components (FIG. 3). These components were subjected to GLC-MS (Table 1). The first fraction (Sh18HF-1) contained glucosamine and small amounts of alanine, glutamic and diaminopimelic and muramic acid, suggesting the presence of cell wall fragments. The second fraction (Sh18HF-2) contained disaccharides: 2-acetamido-2-deoxy-hexosylribitol (pseudomolecular ion [M-1].sup.+ of 356) and 2-acetamido-2-deoxy-hexosylglycerol (pseudomolecular ion [M-1].sup.+ of 296) as determined by FAB-MS analysis. GLC-MS analysis revealed peracetylated glycerol, ribitol, 2-acetamido-2-deoxy-galactose and 2-acetamido-2-deoxy-glucose in the molar ratio presented in Table 1. Methylation analysis performed with GLC-MS detected 4-O-acetyl-1,2,3,5-tetra-O-methyl-ribitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-2-N-methylacetamido-2-deoxy-glucit- ol as major components, indicating the substitution of ribitol by GlcNAc on carbon C-4. Free ribitol and glycerol were recovered in fraction Sh18HF-3.

Detail Description Paragraph:
[0120] In the next step, oxidation of the Sh18 CWP with periodate was performed, and the products were separated on a BioGel P-60 column and analyzed (FIG. 4). No ribitol was detected, indicating that it had been completely oxidized. The composition of the two major fractions eluted from the column (Sh18-oxid-1, Sh18-oxid-2) is shown in Table 1. GLC-MS and NMR analysis of Sh18-oxid-1 demonstrated the presence of 1,3-poly(glycerol phosphate). The low-molecular-mass fraction Sh18-oxid-2 was composed of glycerol and .beta.-GlcNAc phosphate, representing the degradation products of the minor polymer: ->3-O-.beta.-GlcNAc-(1->- 4)-ribitol-1-OPO.sub.3.

Detail Description Paragraph:
[0121] In order to examine whether the polymers were bound to each other by the cell wall fragments, the Sh18 CWP was treated with 10 mM HCl, as mild acid hydrolysis has been reported to cause cleavage of the linkage between the cell wall polysaccharide and the phosphate on the muramic acid in the peptidoglycan moiety (Argman et al. (1985) J Bacteriol 161, 299-306). The product of mild acid hydrolysis was eluted from a BioGel P60 column as a single peak, in the same position as the nontreated material, indicating that it was not affected by this treatment. GLC-MS analysis revealed ribitol, glycerol, and amino sugars in molar ratios similar to those of the original material; also, NMR analysis revealed a similar spectrum.

Detail Description Paragraph:
[0122] Sh18-CWP reacted by double immunodiffusion with anti-Hib and anti-S. epidermidis antibodies with a strong precipitation line and showed a weaker line with anti-S. aureus teichoic acid serum. Immunoelectrophoresis with the intermediate gel containing anti-Hib and the upper gel containing anti-S. epidermidis serum showed that part of the CWP was captured by the anti-Hib, whereas some migrated further and precipitated with the anti-S. epidermidis serum. Similar results were obtained when the order of the antisera in the gels was reversed. These data suggest that at least some of the poly(glycerol phosphate) and poly (ribitol phosphate) chains of Sh18-CWP were not bound. To further address the question of possible linkage between the polymers, Sh18 CWP was precipitated with anti-Hib serum, and the resulting precipitate was washed and analyzed by GLC-MS. The analysis revealed glycerol, ribitol and glucosamine in the ratio: 0.2:1.0:0.1. Without wishing to be bound by any particular type of association, the lesser amount of glycerol than in the original preparation might indicate that some of the poly(glycerol phosphate) may have been free and some may have been connected to the poly(ribitol phosphate). In any case, the precise fashion by which polysaccharides of the invention are associated with one another and/or with proteins or peptides in a glycoconjugate of the invention is presently not viewed as critical.

Detail Description Paragraph:
[0123] B. pumilus Sh 18 CWP NMR spectrum showed characteristic shifts for poly (ribitol phosphate) and poly(glycerol phosphate) as compared to standards (Table 2). The ratio of ribitol carbons to those of glycerol was close to 1:0.5.

Detail Description Paragraph:
[0124] Table 2. .sup.1H-NMR chemical shifts of polysaccharides and their fragments from B. pumilus Sh18, S. epidermidis and S. aureus

Detail Description Paragraph:
[0126] The identity and purity of H. influenzae type a and b CP was evaluated by NMR spectroscopy by comparison of the isolated polysaccharides with the published spectra (Zon et al. (1983), Carbohydr Res. 114, 103-21; Lemercinier et al. (2000), Biologicals 28, 175-83; Branefors-Helander (1977), Carbohydr Res. 56, 117-22). GLC-MS and NMR data obtained for other cell wall polysaccharides of Gram (+) bacteria demonstrated that S. aureus polysaccharide was composed of 1,5-poly(ribitol phosphate) substituted with 2-acetamido-2-deoxy-.beta.-g- lucose on C-2 and S. epidermidis polysaccharide of 1,3-poly(glycerol phosphate) substituted with .alpha.-glucose on C-2. CWP from B. pumilus Sh17 was a mixture of unsubstituted 1,3-poly(glycerol phosphate) and 1,6-poly(2-acetamido-2-deoxy-.alpha.-mannose phosphate) in the molar ratio 1:0.4. These data are in agreement with previous reports (Baddily et al. (1962), Biochem J. 82, 439-448; Endl et al. (1984), Arch Microbiol. 137, 272-80; Vann et al. (1976), Infect Immun. 13, 1654-62). The structures are presented on FIG. 1 and .sup.13C NMR shifts in Table 2.

Detail Description Paragraph:
[0130] The protein/CWP weight ratios in conjugate I and conjugate II were 2.5:1.0

and 2.7:1.0, respectively. The ratios of glycerol, ribitol, GlcNAc and GalNAc, measured by GLC-MS, were 0.45:1.0:0.25:0.11 and 0.50:1.0:0.12:0.08, respectively. .sup.31P NMR analyses of the conjugates showed similar spectra to those of native CWP. Double immunodiffusion analysis of both showed a line of identity with antil-Hib and anti-protein sera. Antibody levels induced by the conjugates and the inhibition by cross-reactive polysaccharides are presented in FIG. 5 and FIG. 6. Conjugate I-induced antibodies reacted with ribitol phosphate-containing polysaccharides of Hib, Hia, and S. aureus and with glycerol phosphate-containing polysaccharides of Sh18, Sh17 and S. epidermidis, whereas conjugate II-induced antibodies reacted only with polysaccharides containing glycerol phosphate. No bactericidal activity against Hib and Hia was detected in sera induced by the conjugates diluted 1:4. Quantitative analysis of these sera assayed in comparison with a monoclonal anti-Hib serum showed levels of anti-Hib antibodies of about 1.0 .mu.g/ml.

Detail Description Table CWU:
1TABLE 1 Molar ratio of B. pumilus Sh18 CWP components and the compositions of fractions obtained after HF and sodium periodate degradation as determined by GLC-MS. GlcNAc: 2-acetamido-2- deoxy-glucose; GalNAc: 2-acetamido-2-deoxy-galactos- e Molar ratio of Sample preparation Glycerol Ribitol GlcNAc GalNAc Sh18-CWP.sup.1 0.43 1.0 0.0 0.0 Sh18-CWP.sup.2 0.45 1.0 0.15 0.06 Sh18-HF-1.sup.3 (11%) 0.0 0.0 1.0.sup.4 0.0 Sh18-HF-2 (57%) 0.6 1.0 0.7 0.30 Sh18-HF-3 (32%) 0.2 1.0 0.0 0.0 Sh18-oxid-1 1.0 0.0 0.08 0.0 Sh18-oxid-2 1.0 0.0 0.7 0.0 .sup.1hydrolysis with HF, .sup.2hydrolysis with HF followed by HCl, .sup.3hydrolysis with HF followed by fractionation on a Bio Gel P-2 column, .sup.4Sh18 HF-1 fraction also contained traces of muramic acid and amino acids detected in separate experiments by GLC-MS

Detail Description Table CWU:
2TABLE 2 .sup.13C and .sup.31P NMR chemical shifts (ppm) of polysaccharides and their fragments from B. pumilus Sh18, S. epidermidis, and S. aureus. S. aureus B. pumilus Sh18 F2/HF F3/HF S. epidermidis Rib-P.sup.a C-1 67.24d 65.65d C-2 71.62d 71.00d C-3 71.93 71.75 C-4 71.62d 80.19d C-5 67.24d 67.41d Gro-P C-1 67.02d 66.06d C-2 70.35t 76.14t C-3 67.02d 65.34d Rib C-1 63.15 C-2 72.87 C-3 72.97 C-4 72.87 C-5 63.15 .beta.-GlcNAc C-1 101.63 102.00 102.12 C-2 55.59d 56.49 56.43 C-3 79.53d 74.52 74.64 C-4 70.36d 70.59 70.66 C-5 75.94 76.50 76.50 C-6 61.35 61.38 61.38 NAc 23.36 23.01 23.20 C.dbd.O 175.54 175.53 175.76 .alpha.-GalNAc C-1 97.76 97.88 C-2 50.52 50.75 C-3 68.46 68.39 C-4 69.34 69.26 C-5 71.85 71.95 C-6 61.98 61.95 NAc 22.84 22.71 C.dbd.O 175.34 175.38 .alpha.-GlcNAc C-1 97.60 97.66 C-2 54.42 54.53 C-3 71.65 71.65 C-4 70.82 70.70 C-5 72.83 72.90 C-6 62.22 61.26 NAc 22.86 22.63 C.dbd.O 175.35 175.21 .alpha.-Glc C-1 98.50 C-2 72.25 C-3 73.72 C-4 73.42 C-5 72.60 C-6 61.33 .beta.-GlcNAc-Rib C-1 67.24d 62.97 C-2 70.85d 71.95 C-3 71.5 72.17 C-4 82.42 82.59 C-5 62.24 61.59 .alpha.-GalNAc-Gro C-1 66.05d 62.09 C-2 76.40t 79.77 C-3 66.05d 61.14 .alpha.-GlcNAc-Gro C-1 65.24d 62.11 C-2 76.46t 79.81 C-3 64.24d 62.14 Rib-P P-1(5) 3.64 3.00 3.24 Gro-P P-1(3) 3.08 3.00 2.45 .beta.-GlcNAc-P P-3 2.59 .alpha.-GalNAc-Gro-P P-1(3) 2.84 .alpha.-GlcNAc-Gro-P P-1(3) 2.84 .sup.aThe suffix P indicates the attachment of a phosphate group to the polyol or amino sugar., nr--not resolved

CLAIMS:

1. A glycoconjuguate preparation comprising one or more polysaccharides derived from a cell wall polysaccharide preparation from B. pumilus Sh 18, wherein one or more of the polysaccharides is bound to a protein or peptide, and wherein the glycoconjugate preparation induces antibodies cross reactive at least with cell wall polysaccharide (CWP) of S. aureus and/or with CWP of S. epidermidis and/or with capsular polysaccharide (CP) of Hia.

3. The glycoconjuguate preparation of claim 2, wherein the glycoconjugate induces antibodies cross reactive at least with CWP of S. aureus and S. epidermidis and with CP of Hia and Hib.

4. The glycoconjugate preparation of claim 1, which comprises a 1,5-poly(ribitol phosphate) and a 1,3-poly(glycerol phosphate).

5. The glycoconjugate preparation of claim 1, which comprises (a) an unsubstituted 1,5-poly(ribitol phosphate); (b) a 1,3-poly(glycerol phosphate); and (c) a poly(2-acetamido-2-deoxy-.beta.-glucosyl-1.fwdarw.4- -ribitol phosphate) with the phosphodiester bonds located between C-1 of ribitol and C-3 of 2-acetamido-2-deoxy-.beta.-glucose.

6. A glycoconjugate preparation consisting essentially of (a) an unsubstituted 1,5-poly(ribitol phosphate) bound to a protein or peptide, or (b) an unsubstituted 1,3-poly(glycerol phosphate) bound to a protein or peptide.

7. A glycoconjugate preparation comprising (a) an unsubstituted 1,5-poly(ribitol phosphate); (b) a 1,3-poly(glycerol phosphate); and (c) a poly(2-acetamido-2-deoxy-.beta.-glucosyl-1.fwdarw.4-ribitol phosphate) with the phosphodiester bonds located between C-1 of ribitol and C-3 of 2-acetamido-2-deoxy-.beta.-glucose, wherein one or more of the polysaccharides is bound to a protein or a peptide.

8. The glycoconjugate of claim 7, wherein about 14% of the 1,3-poly(glycerol phosphate) is substituted by 2-acetamido-2-deoxy-.beta.- -galactose, and/or about 7% is substituted by 2-acetamido-2-deoxy-.alpha.-- glucose, on position C-2.

11. The glycoconjugate preparation of claim 1, wherein one or more of the polysaccharide(s) are bound to peptides or proteins by a linkage of the terminal phosphate group of the polysaccharide to a reactive amino group of the peptide or protein, and wherein the glycoconjugate preparation induces antibodies cross reactive at least with CWP of S. aureus and/or with CWP of S. epidermidis and/or with CP of Hia.

13. The glycoconjugate preparation of claim 12, wherein the glycoconjugate preparation induces antibodies cross reactive at least with CWP of S. aureus and S. epidermidis and with CP of Hia and Hib.

17. The antibody of claim 16, which is cross-reactive at least with CWP of S. aureus and/or with CWP of S. epidermidis and/or with CP of Hia.

19. The polyclonal antibody preparation of claim 18, which is cross-reactive at least with CWP of S. aureus and S. epidermidis and with CP of Hia and Hib.

24. The method of claim 23, wherein the glycoconjugate preparation induces antibodies cross reactive at least with cell wall polysaccharide (CWP) of S. aureus and/or with CWP of S. epidermidis and/or with capsular polysaccharide (CP) of Hia.

26. The method of claim 25, wherein the glycoconjugate preparation induces antibodies cross reactive at least with CWP of S. aureus and S. epidermidis and with CP of Hia, and Hib.

27. The method of claim 22, further comprising detecting resistance of the subject to infection by S. aureus and/or S. epidermidis and/or Hia.

29. The method of claim 22, further comprising detecting resistance of the subject to infection by S. aureus and S. epidermidis and Hia and Hib.

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DOCUMENT-IDENTIFIER: US 6703025 B1
TITLE: Multicomponent vaccines

Detailed Description Text (95):
Teichoic acids, lipoteichoic acid for example, which are polymers of glycerol or ribotol phosphate, are linked to the peptidolglycan and can be antigenic. Antiteichoic antibodies detectable by gel diffusion may be found in patients with active endocarditis due to S. aureus.

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Summary of Invention Paragraph:
[0008] According to the bacterial species, the teichoic acids are of "alcohol polymer type" like in the case of Staphylococcus aureus, or of "polysaccharide type" like in the case of Streptococcus pneumonaie. In this species, two ubiquitous teichoic acids are known: the C-polysaccharide and lipoteichoic acid (also referred to as Forssman antigen).

0181] A cell wall, particularly of Gram-positive Eubacteria, may comprise up to 50% teichoic acid. Teichoic acid is an acidic polymer comprising monomers of a phosphate and glycerol; phosphate and ribitol; or N-acetylglucosamine and glycerol. A sugar (e.g., glucose) and/or an amino acid (e.g., D-alanine) is usually attached to the glycerol or ribitol of a teichoic acid. In addition to direct association with or integration into a cell wall, a teichoic acid may be associated with a phospholipid bilayer adjacent to a cell wall. Often, a teichoic acid is covalently bonded to a glycolipid of a cell membrane, and is known as a "lipoteichoic acid." Teichic acids are common in the genera Staphylococcus, Micrococcus, Bacillus, and Lactobacillus.

3. Endl, J.; Seidl, H. P.; Fiedler, F.; and Schleifer, K. H. 1983. Chemical composition and structure of cell wall teichoic acid of staphylococci, Arch Microbiol, 135: 215-223.

Cell wall teichoic acid, like peptidoglycan, is synthesized at the outer surface of the cell membrane using a nucleotide precursor (CDPglycerol) as the building block. Teichoic acid is a polymer of polyglycerolphosphate that is covalently attached to the peptidoglycan of gram positive bacteria. The enzyme CDP-Glycerol: Poly(glycerophosphate) glycerophosphotransferase catalyzes the polymerization of glycerolphosphate monomers from CDP-glycerol into a chain of polyglycerolphosphate linked via 1,3-phosphodiester bonds. Lipoteichoic acid is a related polymer of polyglycerolphosphate which is anchored to the cell membrane but is not attached to peptidoglycan.

☐ 1: Arch Microbiol. 1983 Sep;135(3):215-23. Related Articles, Links

Chemical composition and structure of cell wall teichoic acids of staphylococci.

Endl J, Seidl HP, Fiedler F, Schleifer KH.

The cell wall teichoic acid structures of 22 staphylococci including 13 type strains were determined. Most of the strains contain a poly(polyolphosphate) teichoic acid with glycerol and/or ribitol as polyol component. The polyolphosphate backbone is partially substituted with various combinations of sugars and/or amino sugars. Most of the substituents occur in a monomeric form but some strains also contain dimers of N-acetylglucosamine as substituents. Staphylococcus hyicus subsp. hyicus NCTC 10350 and S. sciuri DSM 20352 revealed rather complex cell wall teichoic acids. They consist of repeating sequences of phosphate-glycerol-phosphate-N-acetylglucosamine. The amino sugar component is present in this case as a monomer or an oligomer (n less than or equal to 3). Moreover, the glycerol residues are partially substituted with N-acetylglucosamine. The cell wall teichoic acid of S. auricularis is a poly(N-acetylglucosaminyl-phosphate) polymer similar to that found in S. caseolyticus ATCC29750. The cell wall teichoic acid structures for type strains of S. auricularis, S. capitis, S. cohnii, S. haemolyticus, S. hominis, S. hyicus subsp. hyicus, S. sciuri, S. xylosus and S. warneri were determined for the first time in detail. The structures of some of the previously described teichoic acids had to be revised (S. epidermidis, S. simulans, S. aureus phage type 187).

PMID: 6639273 [PubMed - indexed for MEDLINE]

DOCUMENT-IDENTIFIER: US 6428971 B1
TITLE: Teichoic acid enzymes and assays

Detailed Description Text (20):
The biosynthetic pathway for teichoic acid has been established for many years, yet the exact function of this anionic polymer has never been determined. One report describes the use of teichoic acid as a reserve phosphate source in which gram positive bacteria draw upon the glycerolphosphate when phosphate levels in the environment are low (Grant W D. "Cell wall teichoic acid as a reserve phosphate source in Bacillus subtilis" J Bacteriol (1979) vol. 137, pp. 35-43, incorporated by reference). While this role for teichoic acid cannot be disputed, the fact that B. subtilis cannot survive in the absence of teichoic acid synthesis under conditions of high phosphate levels (Mauel C, Young M, Margot P, Karamata D. "The essential nature of teichoic acids in Bacillus subtilis as revealed by insertional mutagenesis" Mol Gen Genet (1991) vol. 215, pp. 388-394, incorporated by reference) indicate that a more essential role is likely. Some reports point to the ability of teichoic acid to chelate divalent cations (Fischer, W. "Lipoteichoic acid and lipids in the membrane of Staphylococcus aureus" Med. Microbiol. Immunol. (1994) vol.183, pp. 61-76, incorporated by reference), but lipoteichoic acid would presumably chelate in the absence of cell wall teichoic acid. It is far more likely that the essential nature of teichoic acid is in maintaining the structural integrity of the cell wall, due to the covalent attachment to peptidoglycan (FIG. 8). Given the information disclosed herein it would be obvious to one skilled in the art to randomly mutate the cloned rodC gene, integrate the mutated gene back into the chromosome, and produce a pool of TAP mutants which can be used to study the effects of teichoic acid on gram positive cell wall integrity.

Detailed Description Text (31):
Preliminary experiments demonstrated that incubation of lipoteichoic acid and CDP[.sup.3 H]glycerol with TAP resulted in the formation of an acid precipitable material. When this material was analyzed by cellulose thin-layer chromatography, it remained at the origin, indicating that a high molecular weight compound had been formed in the TAP assay. Hydrolysis of this product at 100.degree. C. in the presence of 1 N HCl resulted in the formation of degradation products which comigrated with glycerol and glycerol-3-phosphate. The chromatography profile matches that which has been reported for the acid catalyzed degradation of polyglycerol-phosphate (Burger M M, Glaser L. "The synthesis of Teichoic Acids" J. Biol. Chem. (1964), vol. 239, pp. 3168-3177, incorporated by reference) the data demonstrate that lipoteihoic acid can serve as an acceptor for the transfer of [.sup.3 H]glycerol-3-phosphate from CDP[.sup.3 H]glycerol in the TAP catalyzed reaction thereby lengthening the polyglycerol-phosphate chain. Table 2 shows that commercial lipoteichoic acid preparations from S. aureus and S. faecalis can also serve as acceptors for the transfer of [.sup.3 H]glycerol-3-phosphate from CDP[.sup.3 H]glycerol.

Detailed Description Text (33):
This assay is based on the ability of lectins such as wheat germ agglutinin (WGA) and concanavalin A (conA) to bind the sugar moieties present on lipoteichoic acids isolated from a variety of gram positive bacteria. For example, the enzyme can be mixed with buffer, [.sup.3 H]CDP-glycerol, and 10 .mu.g of Enterococcus faecalis lipoteichoic acid as described for the precipitation assay above. After incubating at 37.degree. C. for 1 hour, streptavidin SPA beads (Amersham) containing biotinylated concanavilin A are added to the assay and the entire mix is incubated at room temperature for 30 min. in a 96 well plate. The conA::SPA bead conjugate will bind the radioactive lipoteichoic acid formed in the assay and the activity of the enzyme can be quantitated using a counter such as a Packard Top Counter. A variety of lipoteichoic acids can serve as substrates and the appropriate lectin can be bound to a SPA bead. For example, the glucose moieties present on the lipoteichoic acids of Enterococcus faecalis, Enterococcus faecium, and Enterococcus hirae can be bound to SPA beads containing conA. The cell wall teichoic and

lipoteichoic acids of Staphylococcus aureus containing N-acetylglucosamine residues can be bound to WGA beads.

CLAIMS:

1. A process for determining teichoic acid polymerase activity in a sample, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and the sample to form a mixture; incubating the mixture under conditions effective for a teichoic acid polymerase in the sample, to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein, wherein the teichoic acid polymerase is encoded by DNA having at least about 70% homology with SEQ ID NO:1; and determining the presence or absence of the lipoteichoic acid product.

9. A process for determining the presence or absence of lipoteichoic acid from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis in a sample, the method comprising: combining CDP-glycerol, water, a teichoic acid polymerase encoded by DNA having at least about 70% homology with SEQ ID NO:1, and the sample to form a mixture; incubating the mixture under conditions effective for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into a lipoteichoic acid present in the sample to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product.

17. A process for screening teichoic acid polymerase inhibitors, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, a teichoic acid polymerase encoded by DNA having at least about 70% homology with SEQ ID NO:1, and a teichoic acid polymerase inhibitor to form a mixture; incubating the mixture under conditions effective, when the teichoic acid polymerase inhibitor is not present, for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product produced when the teichoic acid polymerase inhibitor is present in the mixture relative to the lipoteichoic acid product produced when the teichoic acid polymerase inhibitor is not present in the mixture.

22. A process for monitoring enzymatic reactions catalyzed by teichoic acid polymerase, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and a teichoic acid polymerase encoded by DNA having at least about 70% homology with SEQ ID NO:1, to form a mixture; incubating the mixture under conditions effective for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product.

39. A process for determining teichoic acid polymerase activity in a sample, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and the sample to form a mixture; incubating the mixture under conditions effective for a teichoic acid polymerase in the sample to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety

incorporated therein, wherein the teichoic acid polymerase has an amino acid sequence with at least about 70% homology with SEQ ID NO:2; and determining the presence or absence of the lipoteichoic acid product.

43. A process for determining the presence or absence of lipoteichoic acid from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis in a sample, the method comprising: combining CDP-glycerol, water, a teichoic acid polymerase having an amino acid sequence with at least about 70% homology with SEQ ID NO:2, and the sample to form a mixture; incubating the mixture under conditions effective for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into a lipoteichoic acid present in the sample to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product.

47. A process for screening teichoic acid polymerase inhibitors, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, a teichoic acid polymerase having an amino acid sequence with at least about 70% homology with SEQ ID NO:2, and a teichoic acid polymerase inhibitor to form a mixture; incubating the mixture under conditions effective, when the teichoic acid polymerase inhibitor is not present, for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product produced when the teichoic acid polymerase inhibitor is present in the mixture relative to the lipoteichoic acid product produced when the teichoic acid polymerase inhibitor is not present in the mixture.

51. A process for monitoring enzymatic reactions catalyzed by teichoic acid polymerase, the method comprising: combining CDP-glycerol, water, a lipoteichoic acid substrate from a bacterium selected from the group consisting of Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis, and a teichoic acid polymerase having an amino acid sequence with at least about 70% homology with SEQ ID NO:2 to form a mixture; incubating the mixture under conditions effective for the teichoic acid polymerase to cause the incorporation of at least one glycerol-3-phosphate moiety into the lipoteichoic acid substrate to form a lipoteichoic acid product having at least one additional glycerol-3-phosphate moiety incorporated therein; and determining the presence or absence of the lipoteichoic acid product.

L4: Entry 1 of 9 File: PGPB Jul 21, 2005

DOCUMENT-IDENTIFIER: US 20050158346 A1
TITLE: Antimultiorganism Glycoconjugate vaccine

Detail Description Paragraph:
[0122] Sh18-CWP reacted by double immunodiffusion with anti-Hib and anti-S. epidermidis antibodies with a strong precipitation line and showed a weaker line with anti-S. aureus teichoic acid serum. Immunoelectrophoresis with the intermediate gel containing anti-Hib and the upper gel containing anti-S. epidermidis serum showed that part of the CWP was captured by the anti-Hib, whereas some migrated further and precipitated with the anti-S. epidermidis serum. Similar results were obtained when the order of the antisera in the gels was reversed. These data suggest that at least some of the poly (glycerol phosphate) and poly(ribitol phosphate) chains of Sh18-CWP were not bound. To further address the question of possible linkage between the polymers, Sh18 CWP was precipitated with anti-Hib serum, and the resulting precipitate was washed and analyzed by GLC-MS. The analysis revealed glycerol, ribitol and glucosamine in the ratio: 0.2:1.0:0.1. Without wishing to be bound by any particular type of association, the lesser amount of glycerol than in the original preparation might indicate that some of the poly(glycerol phosphate) may have been free and some may have been connected to the poly(ribitol phosphate). In any case, the precise fashion by which polysaccharides of the invention are associated with one another and/or with proteins or peptides in a glycoconjugate of the invention is presently not viewed as critical.

DOCUMENT-IDENTIFIER: US 6923973 B1
TITLE: Peptide and DNA immunization against Coccidioides immitis infections

Detailed Description Paragraph Table (2):
TABLE B Exemplary Adjuvants Alhydrogel Alkyl Lysophosphilipids (ALP) Bcg Bestatin Biliverdin including derivatives and glycoconjugates Bilirubin including derivatives and glycoconjugates, such as monoglycouranoglycans and diglycouranoglycans Biotin including biotinylated derivatives Carnosine including derivatives Chitin Chitosan deacetylated chitin Cholesteryl Succinate Cornyebacterium Parvum whole or part of cell including oligosacchar- ides and glycolipids C. Granulosum whole or part of cell including P40 a peptid- oglycan with a glycoprotein Monophoshoryl Lipid A Deacetylated Monophosphoryl Lipid A Synthetic Isoprinosine Lithosperman lithosperman A, lithospemian B or litho- sperman C Trehalose Monomycolate Trehalose Dimycolate Mycobacterial Species whole or part of Cell including glycolipids, phenolic glycolipids, peptides such as 45/47 kda and BCG Muramyl Dipeptide N-acetyl muramyl-L-alanyl-D-isoglutamine Muramyl Tripeptide MF75.2 Threonyl-Muramyl Di- peptide Murametide Murabutide Lipoteichoic Acid LTA Ribitol Teichoic Acid RTA Glycerol Teichoic Acid GTA Superantigens S. aureus enterotoxins, S. epidermidis enterotoxins, S. pyogenes enterotoxins, E. coli exotoxins Staphylococcus Species whole or part of cell including peptid- oglycans and enterotoxins Viruses whole or part of particle including Vaccinia, Newcastle disease visurs, vesicular stomatitis virus, papilloma virus and rhinovirus Synthetic Peptides pentamers, hexamers, heptamers, octamers, nonamers, decamers, etc.; such as polylysine and threonine-alanine peptides Recombinant Prolactin Glycosaminoglycans and lipid and peptide derivatives Glycosaminoglycourano- glycans Glycosaminoglycolipids Glycosaminoglycourano- Glycolipids Glycosaminoglycopeptides Glycosaminoglycourano- Glycopeptides Phosphorylated Glycosaminoglycans Sulphanted Glycos- aminoglycan Qs-21 Quil-A Polymethylmethyl Acrylate (PMMA) Retinoic Acid Lentinan Levan Malic Anhydride-Divinyl Ether (MVE-2) Hemocyanin from keyhole limpet (KLH) Hemoerythrin molluscan, arthropod hemoerythrin from annelids and lower invertebrates Pteridines Nucleic Acids preferably poly A, poly T, poly AT, poly GC and poly IC-LC Oligonucleotides varying kilobases Lentinen Lectins part or whole; from plants and animals

DOCUMENT-IDENTIFIER: US 6703025 B1
TITLE: Multicomponent vaccines

Detailed Description Text (95):
Teichoic acids, lipoteichoic acid for example, which are polymers of glycerol or ribotol phosphate, are linked to the peptidolglycan and can be antigenic. Antiteichoic antibodies detectable by gel diffusion may be found in patients with active endocarditis due to S. aureus.

TABLE 6

Detection of Methanol-Fixed SA5, SA8 and S. Hay
By Purified Monoclonal Anti-Hay Using Protein A

Anti-Hay Dilution	ATCC SA5	ATCC SA8	USU Hay
500	1.329	3.345	3.017
1000	1.275	2.141	2.266
2000	0.873	1.016	1.487
4000	0.333	0.491	0.951
8000	0.159	0.232	0.490
16000	0.132	0.149	0.331
Normal Mouse 1000	0.101	0.090	0.082
Buffer	0.102	0.113	0.152

Purified anti-Hay stock = 1.63 mg/ml

TABLE 8

Reactivity of Anti-Hay MAB 96-110 on wells Coated with Several LTA's

Antibody ID	Concentration or Dilution	LTA from S. mutans	LTA from S. aureus	LTA from S. faecalis
Buffer	-	0.145	0.172	0.140
Anti-Hay	0.9 ug/ml	3.899	3.253	3.153
MAB	0.3 ug/ml	3.523	2.824	2.769
96-110	0.1 ug/ml	2.023	2.421	2.133
	0.033 ug/ml	2.143	1.590	1.539
	0.011 ug/ml	1.396	0.998	0.832

TABLE 9

Inhibition of Anti-Hay MAB 96-110
with LTA From Different Gram Positive Bacteria

LTA Inhibitor (ug/ml)	LTA S. mutans	LTA S. aureus	LTA S. faecalis
9	0.298	0.360	0.140
3	0.449	0.434	0.496
1	0.549	0.538	0.545
0.37	0.558	0.526	0.549
0.12	0.509	0.735	0.582
0.04	0.574	0.614	0.671
0	0.621	0.607	0.648

NOTES:

1. Wells were coated with methanol-fixed Hay.
2. Wells were blocked with 1% BSA in PBS.
3. Monoclonal anti-Hay was used at a final concentration of 0.5 ug/ml and reacted with inhibitors at the concentrations indicated in the Table.
4. Detection was with a gamma-specific Rabbit anti-Mouse.
5. Substrate was TMB.

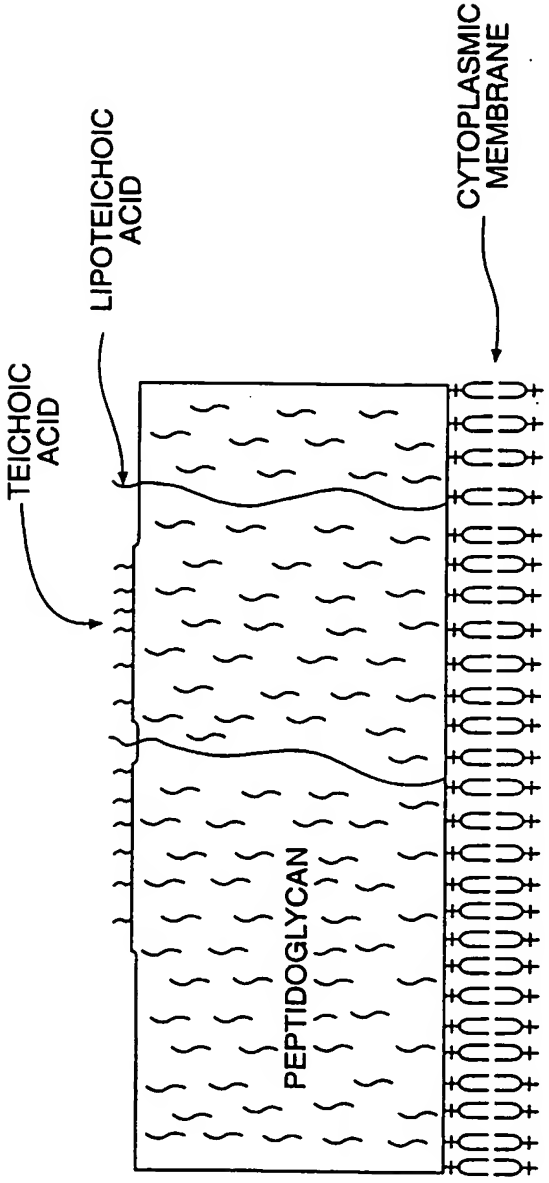
CYTOPLASMIC MEMBRANE
LIPOTEICHOIC ACID
TEICHOIC ACID
PEPTIDOGLYCAN

FIG. 1